Message

From: Strynar, Mark [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=5A9910D5B38E471497BD875FD329A20A-STRYNAR, MARK]
Sent: 5/1/2018 11:58:31 AM
To: Nadine Kotlarz [nkotlar@ncsu.edu]
CC: McCord, James [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=McCord, James]
Subject: RE: Update on serum method

I had to take the day off for a kitchen remodel painting session at home. I am in today and glad to discuss. I think the bigger issue is figuring out the system contamination issue so we can measure for what we actually see in the serum samples. Everything else is secondary.

Mark

From: Nadine Kotlarz [mailto:nkotlar@ncsu.edu]
Sent: Monday, April 30, 2018 9:58 AM
To: Strynar, Mark <Strynar.Mark@epa.gov>
Cc: McCord, James <mccord.james@epa.gov>
Subject: Fwd: Update on serum method

Good morning,

FYI See below some suggestions from Andy.

I'm at EPA today. Can we speak about next steps to handle the legacy PFAS contamination on the Orbitrap?

Nadine

---------- Forwarded message ----------
From: **Lindstrom, Andrew** <Lindstrom.Andrew@epa.gov>
Date: Mon, Apr 30, 2018 at 9:07 AM
Subject: RE: Update on serum method
To: Nadine Kotlarz <nkotlar@ncsu.edu>
Cc: "Detlef R. U. Knappe" <knappe@ncsu.edu>

Nadine,

Thank you very much.

I'm wondering if GenX is going to be in the blood at all. It would be a shame to work very hard to develop a method for something that doesn't happen (at least in this cohort).

This is something the CDC (Antonia) has consistently commented on. The NHANES programs forces them to try to quantitate shorter PFAS in serum when they almost always get <LOD and they know it is coming out in the urine. She says they waste a lot of time and money looking for stuff that won't be there most of the time.

For a lot of these Chemours compounds the urine is probably going to be the key diagnostic biofluid.

If you all took a peak at some of the urine samples you might get some reassurance that you are not missing a big part of the story.

Thank you,

Andy

From: Nadine Kotlarz [mailto:nkotlar@ncsu.edu]
Sent: Thursday, April 26, 2018 6:02 PM
To: McCord, James <mccord.james@epa.gov>
Cc: Detlef R. U. Knappe <knappe@ncsu.edu>; Jane Hoppin <jahoppin@ncsu.edu>; Lindstrom, Andrew <Lindstrom.Andrew@epa.gov>; Strynar, Mark <Strynar.Mark@epa.gov>

Subject: Re: Update on serum method

James, Mark,

Here are some questions/comments about the serum method that came up during GenX study investigators meeting today:

1. We should resolve the legacy contamination in the Orbitrap before proceeding with analyzing more samples because we need to be confident in PFOS and PFOA results for comparison with other studies. Do you think it is worth asking Thermo for help?

2. Did we see PFHxA in the serum and/or blanks?

3. Why is the MS response so different between Nafion bp1 and Nafion bp2?

4. Consider switching the column to address PFMOAA eluting in the dead volume, or consider running the samples on the triple quad for PFMOAA analysis since the baseline is lower for that instrument (PFMOAA elutes early in the triple quad method as well). We may need multiple methods to analyze for all of the PFAS.

5. We could proceed with the serum method as is to analyze all samples for GenX and the other compounds. The advantages of the method are that it's fast and it seems to work well for some compounds. If we continue to not see any GenX in the serum, we could choose a subset of samples to try to increase sensitivity to confirm that GenX is not present (e.g., try adding 100 uL serum in the method, blowing down the sample, etc.)

What do you think of these ideas?

Nadine

On Thu, Apr 26, 2018 at 12:44 PM, McCord, James <mccord.james@epa.gov> wrote:

> Detlef – We don't really have a matrix interference problem, our background is fairly consistent regardless of the injection. The advantage of the cartridge would only be in concentration.
>
> I am continuing to try to track down the contamination issue for the legacy compounds, although I admit to being somewhat at my wits end with it.
>
> --
>
> James McCord
>
> **From:** Detlef Knappe [mailto:knappe@ncsu.edu]
> **Sent:** Thursday, April 26, 2018 9:36 AM
> **To:** McCord, James <mccord.james@epa.gov>
> **Cc:** Nadine Kotlarz <nkotlar@ncsu.edu>; Jane Hoppin <jahoppin@ncsu.edu>; Lindstrom, Andrew <Lindstrom.Andrew@epa.gov>; Strynar, Mark <Strynar.Mark@epa.gov>
>
> **Subject:** Re: Update on serum method
>
> So need to find the source of the legacy PFAS contamination before we can proceed?
>
> Also, have you tried the Agilent cartridge to see whether it can help with matrix interferences?

And should we look at some whole blood to see whether we can get a GenX signal there?

Detlef

On Thu, Apr 26, 2018 at 7:49 AM, McCord, James <mccord.james@epa.gov> wrote:

Responding to a couple things here for space.

Detlef – We are observing legacy PFAS compounds in our blank samples that I am fairly certain I have isolated to the LC system, separate from the samples themselves. We were able to run blank serum with no detections just a few weeks ago but have picked up contamination in the meantime. The peaks are several million counts, so it vastly outstrips the low levels we are measuring in intensity and kills the sensitivity.

Nadine – Regarding PFMOAA we have a running background from unrelated noise that makes it difficult to see low levels. Further, the retention time is in the instrument dead volume, which means the response is extremely sensitive to matrix effects, but we are able to observe a peak at ~1.2 minutes (see attached). In the blood it is difficult to determine whether any peaks observed in that region are the result of a compound or of noise without MS/MS confirmation, however I did notice a few samples that had minor peaks in that region. Quantitation would be highly suspect without changes to the chromatography and/or an SIL internal standard.

Regarding PFO2HxA, if we extract the masses we observe peaks in blood as well as in the standard calibration curve, but not in SRM1957 (see attached). The elution time is in that heavily noise influenced dead volume region, which strikes me as slightly too early for PFO2HxA and I am inclined to say that we do not observed this compound conclusively.

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James McCord

From: Detlef Knappe [mailto:knappe@ncsu.edu]
Sent: Wednesday, April 25, 2018 10:56 PM
To: Nadine Kotlarz <nkotlar@ncsu.edu>
Cc: Jane Hoppin <jahoppin@ncsu.edu>; Lindstrom, Andrew <Lindstrom.Andrew@epa.gov>; McCord, James <mccord.james@epa.gov>; Strynar, Mark <Strynar.Mark@epa.gov>
Subject: Re: Update on serum method

Thank you, Nadine! Exciting indeed. When you say:

We see significant background for legacy PFAS in the blanks,

do you mean a noisy background from the matrix that interferes with legacy PFAS or legacy PFAS peaks are detected in the blanks?

Best,

Detlef

On Wed, Apr 25, 2018 at 9:30 PM, Nadine Kotlarz <nkotlar@ncsu.edu> wrote:

Hi everyone,

James, Mark and I have been working on the serum method using EPA's Orbitrap.

Our method is 50 uL serum with formic acid denaturation and acetonitrile protein crash. After centrifugation to separate proteins, we load 100 uL of the acetonitrile fraction into an LC vial for analysis. Injection volume is 25 uL.

We prepared standards in calf serum for PFMOAA, GenX, Nafion byproduct 1, Nafion byproduct 2, and the legacy PFAS at concentrations of 0.1, 0.25, 0.5, 1, 2.5, 5, 10, 15, 20, 25 ng/mL for each compound. We ran 10 randomly selected serum samples from the GenX Exposure Study and we ran SRM1957 to see if our measurement of PFAS in this material is close to what others have reported. We did not do internal standard correction for this run but would include internal standards in the future.

Results are summarized in the summary tab of the excel spreadsheet. Calibration curves (with some points omitted for the different compounds) are in the ppt.

Initial observations:

1. GenX standard curve looks okay down to 0.5 ng/mL.

2. Poor response for Nafion byproduct 1 at the lower end of the cal curve (< 10 ng/mL)

3. For GenX and Nafion byproduct 1, the serum sample response is not significantly different from the blanks (calf serum).

4. We do not see PFMOAA in the samples or the standards (James, can you please confirm that we do not see it even in the 25 ng/mL?)

5. For Nafion byproduct 2, the mean concentration across the 10 serum samples is 3.4 ng/mL, and sample response is significantly higher than the blanks. The concentrations in the serum samples ranged from approx. 1-7 ng/mL.

6. Based on area counts, mean response for PFO4DA across the samples is 2 orders of magnitude above the blanks. We did not calibrate for this compound but Mark did get the standard from Chemours so we could reprepare standards with it.

7. Based on area counts, mean response for PFO5DoDA across the 10 samples is one order of magnitude above the blanks. We do not have a standard for this compound.

8. We see significant background for legacy PFAS in the blanks. For now, we do not know how to get rid of it. This background flattens the standard curves for PFHxS and PFOS and makes it difficult to quantify the legacy compounds in the serum samples.

9. In an interlab study of SRM1957, the reference values were 5 +/0 0.40 ng/mL PFOA and 21.1 +/- 1.2 ng/mL PFOS. We measured 4.2 ng/mL and 23.7 ng/mL PFOA and PFOS, respectively.

Please let us know your thoughts. Thanks,

Nadine